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Robyn Jerdan
Scott Cameron
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Robyn Jerdan[1], Scott Cameron[1], Emily Donaldson[1], Olga Iungin[2,3], Olena V. Moshynets[2] & Andrew J. Spiers[1*]

1 School of Applied Sciences, Abertay University, United Kingdom.

2 Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine, Kiev, Ukraine.

3 Kyiv National University of Technologies and Design, Kiev, Ukraine.

* Corresponding Author : Andrew J. Spiers

School of Applied Sciences, Abertay University, Bell Street, Dundee, DD1 1HG, United Kingdom. Tel : +44(0) 1382 308730 Email : a.spiers@abertay.ac.uk

ORCID Author identifiers & Email Addresses –

Scott Cameron	0000-0002-6600-1298	s.cameron@abertay.ac.uk
Emily Donaldson	–	aletajane@btinternet.com
Olga Iungin	0000-0001-8876-6075	olga.suslova11@gmail.com
Robyn Jerdan	0000-0002-7045-8362	1302040@abertay.ac.uk
Olena V. Moshynets	0000-0002-2209-8681	moshynets@gmail.com
Andrew J. Spiers	0000-0003-0463-8629	a.spiers@abertay.ac.uk

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One sentence summary: Serial-transfer of bacterial communities in experimental microcosms alter community-aggregated biofilm traits at the community and individual-strain level and selects for stratified communities with migration recolonising regions after each transfer.

Abstract

The establishment of O_2 gradients in liquid columns by bacterial metabolic activity produces a spatially-structured environment. This produces a high-O_2 region at the top that represents an un-occupied niche which could be colonised by biofilm-competent strains. We have used this to develop an experimental model system using soil-wash inocula and a serial-transfer approach to investigate changes in community-based biofilm-formation and productivity. This involved ten transfers of mixed-community or biofilm-only samples over a total of 10–60 days incubation. In all final-transfer communities the ability to form biofilms was retained, though in longer incubations the build-up of toxic metabolites limited productivity. Measurements of microcosm productivity, biofilm-strength and attachment levels were used to assess community-aggregated traits which showed changes at both the community and individual-strain levels. Final-transfer communities were stratified with strains demonstrating a plastic phenotype when migrating between the high and low-O_2 regions. The majority of community productivity came from the O_2-depleted region rather than the top of the liquid column. This model system illustrates the complexity we expect to see in natural biofilm-forming communities. The connection between biofilms and the liquid column seen here has important implications for how these structures form and respond to selective pressure.

Introduction

Community-based polymicrobial (or multispecies) biofilms are of increasing interest to agricultural, environmental, food, medical and biotechnological microbiologists. Ecological and evolutionary processes are known to effect community structure and productivity in response to natural and anthropogenic pressures (Kaltz *et al.* 2012; Lawrence *et al.* 2012; Fienga *et al.* 2015; Castledine *et al.* 2019). These processes also limit our ability to produce and control multispecies biofilms in a variety of contexts (e.g. treatment of infections, bioremediation, biocontrol and plant growth, wastewater treatment and biomass conversion, etc.). Although single-species biofilms have been extensively characterised, multispecies biofilms are considerably more complex and experimentally more difficult to investigate (it is important to realise that populations and communities behave differently, Tan *et al,* 2017). One approach has been to use artificial bacterial assemblages but the species richness of multispecies biofilms is considerably higher (Tan *et al,* 2017). In these there are far more interactions effecting community productivity, change and resilience, and there is a need to understand such complex biofilms from an ecological perspective (Battin *et al,* 2007; Flemming *et al,* 2016; Nadell *et al*, 2016). Experimentally it is also difficult to replicate the natural conditions biofilms develop in, but simple experimental microcosms providing novel (artificial) environments can be used to investigate the impact of resource availability and disturbance in these communities.

Experimental microcosms have been used to investigate the eco-evolutionary processes driving adaptive radiation and air-liquid (A-L) interface biofilm-formation (Moshynets & Spiers, 2016) for several model bacteria, including *Pseudomonas fluorescens* SBW25 (Koza *et al.* 2017). In statically-incubated liquid microcosms wild-type SBW25 colonists rapidly establish an O_2 gradient that divides the liquid column into an upper high-O_2 region and a lower O_2-depleted (low-O_2) region and produces a spatially-structured environment (Koza *et al.* 2011). The high-O_2 region is known as the Goldilocks zone (Kuśmierska & Spiers 2016; Koza *et al.* 2017) and provides an ecological opportunity for A-L interface biofilm-forming adaptive mutants such as the Wrinkly Spreader which out-compete the non-biofilm–forming ancestral strain in this otherwise O_2 growth-limited system. This type of biofilm-formation is commonplace within soil and plant-associated pseudomonads (Ude *et al.* 2006) and SBW25 biofilms have been visualised on plant roots (Humphris *et al*, 2005; Downie *et al.* 2014). It appears that the cooperative effort needed to produce these structures is a more efficient strategy for colonising the high-O_2 region than the constant aerotaxis needed by individual cells to maintain position and counter the effects of cell diffusion and physical disturbance (Jerdan *et al.* 2019).

O_2 gradients also stratify and define microbial community function in a range of natural environments (Fenchel & Finlay, 2008; Brune *et al*. 2000). Communities established from environmental samples or artificial assemblages of strains in experimental microcosms develop similar stratification, with stochastic and deterministic processes (Liu *et al*. 2019) including ecological filtering/species sorting affecting community coalescence and subsequent change. In the context of SBW25 spatially-structured static microcosms, the high-O_2 region represents an un-occupied niche ready for colonisation by biofilm-competent or aerotaxic community members rather than an ecological opportunity in the sense of adaptive radiation (Wellborn & Langerhans, 2014). We expect that communities established from environmental samples in the same type of static microcosms used for SBW25 would develop O_2 gradients as rapidly as SBW25 colonists (Koza *et al.* 2011; Loudon et *al*. 2016) and also produce A-L interface biofilms. In such community microcosms, we hypothesize competition for limited resources will drive changes in community structure and biofilms and maximise productivity through an increase in the abundance of successful competitors and a reduction in functional redundancy (Nadell *et al.* 2009; Cavaliere *et al.* 2017) (even in a two-species system, interactions can affect productivity, Zhang *et al.* 2012, and biofilm rheology is determined by the fastest-growing member, Abriat *et al.* 2020). However, it is unclear whether the successful colonisation of the relatively-shallow high-O_2 region with a biofilm would contribute more to total microcosm productivity than the colonisation of the low-O_2 region (in SBW25 microcosms the low-O_2 region occupies more than 90% of the liquid column; Koza *et al.* 2011).

Here we investigate the impact of O_2 availability and time limitations on multispecies biofilm-formation and productivity using a serial-transfer experimental microcosm approach with communities established from soil-wash inocula (a defined, natural model system after Tan *et al.* 2017). This provides a mixed assemblage of colonists (though less diverse than soil aggregate samples, Howard *et al*. 2017) and will

include fast-growing aerobic and micro-aerobic pseudomonads, many of which we expect to be biofilm-competent following an earlier survey of environmental pseudomonads (Ude *et al.* 2006). In our closed system, the regular transfer of community samples to the next microcosm represents pulse disturbance/dispersal/re-seeding events from which communities recover, though ecological filtering and competition during each growth period might alter community productivity, structure and community-aggregated traits such as biofilm-formation. Communities were serially-transferred under conditions favouring biofilm–formation or not (i.e. static and longer incubations *vs.* shaken and shorter incubations), with inocula sampled from mixed-communities or directly from the biofilms themselves. We expected that productivity would increase as communities changed to fit these new conditions largely through resource competition (Bachmann *et al*. 2016; Hodapp *et al.* 2019). This would also be reflected in changes in biofilm-formation and the relative contribution of the high and low-O_2 regions to total productivity. Optical density (OD_{600}) measurements were used to follow productivity (Fiegna *et al.* 2015) and the combined biofilm assay (CBA, Robertson *et al.* 2013) to determine changes in community-aggregated traits at the community and individual-strain level (Fierer *et al.* 2014). Cell-localisation experiments (Jerdan *et al*. 2019) were also used to investigate community stratification, strain migration and productivity in the high and low-O_2 regions of our experimental microcosm system.

Materials and Methods

Soil-wash inocula and bacterial strains

Soil-wash inocula were prepared by adding 200g soil sampled from a grass lawn in Dundee (October 2017) with 900ml of sterile water and mixing overnight. This was allowed to settle and 15% (v/v) glycerol added to the cell suspension which was aliquoted and stored at -80°C to provide standardised inocula for experiments. When required, 100µl of thawed suspension was used to inoculate a microcosm which was incubated overnight with shaking for further experimentation. Strains were isolated from soil-wash inocula and final-transfer community samples and stored at -80°C as glycerol stocks (see **Supplementary Information** for more details). *Pseudomonas fluorescens* SBW25 (Bailey & Thompson 1992; Rainey & Bailey 1996) was used as a model pseudomonad in some assays.

Microcosms and culturing conditions

Bacterial strains and communities derived from soil-wash inocula were cultured in microcosms (30ml loosely-lidded glass vials) containing 6ml modified King's B medium (KB*; 1.5% (w/v) agar added for plates; Kuśmierska & Spiers 2016) that were incubated at 20°C with shaking or statically under normal O_2 conditions, or statically under very-low O_2 conditions produced using Oxoid AnaeroGen sachets (Thermo Scientific, UK). The high-O_2 region in static microcosms was visualised with Methylene blue

(Jerdan *et al*. 2019). Autoclaved cell-free culture supernatants from pooled six-day shaken community test microcosms and fresh KB* were used to produce mixed-media and KB* diluted-media prepared with sterile water.

Serial-transfers and community productivity measurements

A serial-transfer experiment with communities established with soil-wash inocula was undertaken with nine treatments as shown in **Supplementary Figure S1**. Replicate communities (*n*=3) were transferred ten times across eleven microcosms with either a one, three or six-day shaken or static incubation period between each transfer. 100µl samples were taken from vigorously-mixed microcosms to sample the entire community, or from the A-L interface biofilm using a 10µl wire-loop which was then vigorously mixed in 1ml sterile water to provide 100µl samples for transfer. At each transfer, replicate microcosms were established (*n*=8), and after incubation, one was sampled for the next transfer and the others assessed for microcosm productivity by measuring optical density (OD_{600}; Fiegna *et al.* 2015) as part of the CBA (see below). Cumulative OD_{600} measurements were also used to estimate the number of 'community' generations passed in each treatment of the serial-transfer experiment (Fiegna *et al.* 2015) and the effective population size estimated using a transfer inocula of ~10^8 cells and 5–6 generations per transfer microcosm (Van den Bergh *et al*. 2018).

Biofilm and microcosm-based assays, strain phenotypes, pair-wise interactions, community richness and diversity indices

The combined biofilm assay (CBA, Robertson *et al.* 2013) was used to measure microcosm productivity (OD_{600}), biofilm-strength (grams) and attachment levels (A_{570}) in static microcosms (*n*=8) after three days. Cell distributions (relative OD_{600}) through the liquid column were assessed in static microcosms (*n*=5) after 24h (Jerdan *et al*. 2019). Transplant tests used biofilm samples, collected with a wire loop and 100µl samples taken mid-column from static microcosms, which were then transferred to fresh microcosms (*n*=5) and incubated statically for three days before determining productivity (OD_{600}) and biofilm-strengths (g). Productivity (change in OD_{600}) under different oxygenation conditions was determined after 24h incubation of shaken, static and very-low O_2 microcosms (*n*=3). The effect of aged culture media on growth (OD_{600}) after 24h was assessed using mixed and diluted-KB* media in shaken microcosms (*n*=5). Strain phenotypes were determined using a variety of growth-based and behavioural assays (Robertson *et al*. 2013). Pair-wise competitive and antagonistic interactions were investigated on KB* plates and incubation for two days before inspection. Categorical phenotype data, as well as CBA strain data, were used to determine community richness, Shannon and Inverse Simpson's diversity indices. See **Supplementary Information** for more assay details.

Statistical analyses

Experiments were performed with replicates (*n*) and data examined using JMP v12 and v14 trial statistical software (SAS Institute Inc.) (*P* values are all rounded up to two decimal places). One-way analysis of variance (ANOVA) and Kruskal-Wallis (Rank Sums) tests with post hoc Tukey-Kramer HSD tests, comparisons with a control using the Steel method and Kruskal-Wallis tests were used to determine differences between treatments. Productivity was investigated using complete data sets with mixed-effects models a Standard Least Squares approach and the Restricted Maximum Likelihood method with unbounded variance components. These included fixed and random effects which were investigated using LSMeans Differences Student's *t* and Tukey HSD tests. Outcomes were further confirmed by re-running models after inspection of residuals and the removal of outliers to ensure Normality determined by Aderson-Darling tests (model details are provided in the **Supplementary Information**). Pearson's (*r*) and Spearman's ρ correlations, Chi-square tests of independence (via www.socscistatistics.com) and odds ratios (via www.medcalc.org) were used to investigate associations, and Principal components analysis (PCA) of correlations was used to visualise data.

Results and Discussion

Experimental approach

We designed a serial-transfer experiment and nine treatments using soil-wash inocula and liquid microcosms to investigate the impact of O_2 availability and time limitations on community-based (multispecies) biofilm-formation and productivity (see **Supplementary Information** for further details of our approach). O_2 gradients were rapidly established in static microcosms with the high-O_2 region detectable within 3h (**Supplementary Figure S2**). For convenience, we refer to the final-transfer communities produced by each of the treatments tested here as the one, three and six-day ShC (*sh*aken incubation with mixed *c*ommunity sample transfer), StC (*st*atic incubation with mixed-*c*ommunity sample transfer) and StB (static incubation with *b*iofilm-only sample transfer) communities.

We ran our serial-transfer experiment over a total of 10–60 days of incubation. This should be sufficient to see differences develop as evolutionary and ecological processes have been observed over relatively short time-scales in other serial-transfer experiments (e.g. Kaltz *et al.* 2012; Lawrence *et al.* 2012; Fienga *et al.* 2015; Castledine *et al.* 2019). In our experiment, communities developed from the initial soil-wash inocula over 49–56 community generations as determined from cumulative OD_{600} measurements (Fiegna *et al.* 2015), and most static microcosms showed signs of biofilm-formation after each incubation and no community replicates were lost due to failed transfers. We determined an estimated effective population size of $5x10^8$ (Van den Bergh *et al*. 2018) with productivity cycling between 0.05–2.3 OD_{600} during the incubation periods. Variations in colony morphology on KB* plates spread with soil-wash inocula and final-transfer community samples confirmed community diversity.

Serial-transfer treatments effect community productivity

Preliminary analysis of productivity (OD_{600}) data from the serial-transfer experiment suggested significant changes had occurred between the first and final incubations of the ShC, StC and StB communities when compared by incubation period (1, 3 & 6 days; **Figure 1;** see **Supplementary Figure S3** for productivity trends across all transfers). In order to identify the factors impacting on productivity across all microcosm transfers and treatments, we employed a mixed-effects modelling approach that identified incubation period (1, 3 & 6 days), conditions (shaken & static) and shift (from the first to the last incubation) as significant effects (P=0.01; see **Supplementary Information** for **Model 1** details). Further investigation of effects showed that, over-all, productivity was 1.3x higher in microcosms incubated for longer periods (OD_{600}: 1 day, 1.48±0.02 (standard error of the mean); 3 days, 1.93±0.01; 6 days, 1.96±0.02; α=0.05) and 1.1x higher in shaken microcosms than in static ones (shaken, 1.89±0.02; static, 1.74±0.02; α=0.05). This suggests that the ecological opportunity offered by longer incubations and greater O_2 availability are significant factors effecting productivity in this serial-transfer system. Sample type (mixed-community & biofilm-only) was only significant in an interaction with incubation period (α=0.05) which suggests that there may be some impact on productivity as a result of the transfer of mixed-community or biofilm-only samples between microcosms.

Further analysis showed that incubation period and conditions impacted on productivity in the first incubation as expected, as well as in the last incubation of the serial-transfers (P=0.01; but not sample type, P=0.91), suggesting that the communities had not fully adapted to the altered growth conditions by the end of the experiment (see **Supplementary Information** for **Model 2** in which all incubations were investigated). However, although productivity increased in many treatments, productivity during the last incubations had fallen in each of the six-day ShC community replicates and in 2/3 of the six-day StC and StB community replicates (**Figure 1**). Inspection of all transfer incubations found negative correlations between productivity and transfer number for 17/27 of the community replicates (ρ=-0.3– -0.15; P=0.01–0.02). Further tests suggest that toxic metabolites were inhibiting growth more quickly than the reduction of nutrients during the serial-transfers (**Supplementary Figure S4**). This is an example of the tragedy of the commons (Estrela *et al.* 2019) and the effects of toxic metabolites on growth can be complex (Travisano, 1997). Further metabolomic analysis would be required to identify the key toxins accumulating here (e.g. Tyc *et al.* 2017), and it would be interesting to determine whether they are normal metabolites that might be toxic to some strains, or whether some strains are producing specific anti-competitive compounds in this system (Hibbing *et al*. 2010).

Changes in community-aggregated traits associated with biofilm-formation

In order to determine whether changes had occurred in community-aggregated traits during the serial-transfer experiment, we used principal components analysis to visualise community CBA data which included measurements of microcosm productivity (OD_{600}), biofilm strength (g) and attachment (A_{570})

for the first and last incubations (**Figure 2**). This suggested that shifts had occurred, especially in the three and six-day treatments, and a mixed-effects model of productivity using the first and last incubation data confirmed biofilm strength and attachment, incubation period (1, 3 & 6 days) and conditions (static & shaken), and shift (between first and last incubations) were significant effects (P=0.01–0.02) but not sample type (mixed-community & biofilm; P = 0.98; see **Supplementary Information** for **Model 3** details). Across all static incubations positive correlations were found between productivity and strength (ρ=0.36, P=0.01) and productivity and attachment (ρ=0.25, P=0.01). Strong biofilms were also well-attached structures (ρ=0.45, P=0.01), and both productivity and biofilm-formation also increased with incubation period (incubation period and productivity, ρ=0.67, strength, ρ=0.65, and attachment, ρ=0.49; P=0.01). These findings all demonstrate that community-aggregated traits changes had occurred during the serial-transfer experiment. We were therefore interested to see if this was also reflected in altered community structure and individual strain characteristics.

Community changes are reflected by differences in community members

We investigated the phenotypes of 240 strains recovered from the soil-wash inocula and one, three and six-day ShC, StC and StB final-transfer communities (n=24 from each) to determine whether the changes in community-aggregated traits measured at the community level were mirrored by changes at the individual-strain level. We determined strain phenotypes, using a range of growth-based and behavioural assays as well as the CBA in test microcosms, which then allowed us to assess community richness and abundance. Diversity indices were the highest for the soil-wash inocula and six-day ShC final-transfer community (TK-HSD, α=0.05; **Supplementary Figure S5**). This suggests that changes in richness and abundance had occurred in the static-microcosm serial-transfers, though no significant correlations were found between community productivity and richness (ρ, richness determined by growth & behaviour assays, P=0.07; by combined biofilm assay, P=0.33). 16S rRNA amplicon and metagenomic sequencing across serial-transfer microcosms could uncover further trends in community change and community-aggregated traits (Fierer *et al*. 2014) and identify strains occupying the high and low-O_2 regions with particularly competitive abilities.

We also modelled the strain productivity data from the CBA using a mixed-effects model that identified strain origin and biofilm attachment as significant effects (P=0.01), but not biofilm strength (P=0.51; see **Supplementary Information** for **Model 4** details). Further investigation of origin effects showed that the soil wash isolates could be differentiated from all but the one-day StB strains (α=0.05), and these differences could also be visualised by principal components analysis (**Figure 3**). Productivity, biofilm strength and attachment were positively correlated (ρ=0.30–0.38, P=0.01) which suggests that faster-growing strains produce stronger and better-attached biofilms (such correlations have been seen for other strains but do not hold in all cases; Ude *et al*. 2006; Robertson *et al.* 2013). This analysis demonstrates that significant changes in community-aggregated traits at the individual-strain level had also occurred

during the serial-transfer experiment. Finally, odds ratios showed that biofilm-forming strains were 4 – 7x more likely to be found in the six-day StC and StB final-transfer communities than in the soil-wash inocula (χ^2, P=0.02–0.06) which suggests that these communities have also retained a degree of functional redundancy. It would be interesting to compare the genomes of these strains to determine whether they were capable of expressing extracellular polymeric substances (EPS) associated with biofilm–formation (Flemming *et al.* 2016) and the range of substances expressed within each community.

We were interested to see if there was evidence of competitive or antagonistic interactions between strains from the soil-wash inocula and six-day final-transfer communities. Although overall more neutral interactions were found in pair-wise assays, competitive interactions were more common than antagonistic interactions (**Supplementary Figure S6**), and a significant dependency was observed between strain origin (soil wash, six-day ShC, StC & StB) and thee type of interaction (antagonistic, competitive & neutral; X^2, P=0.01). Odds ratios suggest that it was 4x more likely to find antagonistic and competitive interactions rather than neutral interactions, between the six-day StC and StB strains tested against the soil wash strains, compared to the six-day ShC strains (X^2, P=0.01), with no significant difference between the six-day StC and StB strains (X^2, P=0.29). As neutral interactions were more common, we predicted that the productivity of static test microcosms inoculated with a mix of strains (n=1, 2, 4 & 8) should not be significantly different from microcosms inoculated with the corresponding community sample. In tests using one and three-day StB strains and community samples, no significant differences were observed between the strain mixtures and community samples (Steel, P=0.24–0.96; **Figure 4A & 4B**), but antagonistic and competitive interactions might be responsible for reducing the productivity we observed in microcosms inoculated with six-day StB community samples (P=0.01; **Figure 4C**). A range of productivity-richness or diversity relationships have been reported for microbial systems (Smith, 2007), and cooperation or reduced competition for resources may not always play important roles in maximising productivity for simple communities with few members (Zhang *et al*. 2012), but competition and antagonistic interactions may start to limit productivity in more complex communities where interactions are likely to occur at the micro-scale within aggregates (Cordero & Datta, 2016).

Community stratification between the high and low-O_2 regions

In our characterisation of strains isolated from the soil-wash inocula and final-transfer communities, we noticed substantial growth occurring below the biofilm in the liquid column in static test microcosms which suggests that the low-O_2 region may contribute significantly to microcosm productivity. In order to better understand this, we investigated cell-distributions down through the liquid column and migration between the high and low-O_2 regions of static test microcosms. Communities were vertically stratified (TK-HSD, α=0.05; **Figure 5)**, with six-day StC and StB final-transfer communities having the

highest level of enrichment at the top of the liquid column and the soil-wash inoculum and the ShC final-transfer community having the lowest (T-K HSD, α=0.05; **Supplementary Figure S7**), whilst mid-column distributions showed the opposite trend (r=-0.99, P=0.02). Enrichment at the top and mid-column distributions were also negatively correlated for six-day StB strains (n=8 tested, ρ=-0.88, P=0.01) but not for six-day ShC and StC or soil-wash inoculum strains (P=0.18, 0.53, 0.82; see **Supplementary Figure S8** for strain cell-distributions).

Although we presume that most cells found mid-column are free swimming 'planktonic' cells, this region may also contain sinking biofilm fragments, and note that for some bacteria cells grow in small aggregates even in liquid culture (Schleck *et al.* 2009). Bioconvection currents may further help circulate cells between the A-L interface and the liquid columns (Hill & Pedley, 2005). Higher levels of enrichment at the top across final-transfer communities compared with the soil-wash inoculum suggest niche selection for strains able to compete efficiently in high-O_2 conditions has occurred, underlying the fact that in our model system O_2 is growth-limiting in both static and shaken microcosms. Ecological filtering has not been strong enough to reduce colonisation of the low-O_2 region, despite the fact that biofilms are known to steepen the O_2 gradient formed in static microcosms (Koza *et al.* 2011; Loudon *et al.* 2016). This may be because many strains are able to compete effectively in both regions because sufficient O_2 remains in this region to support some level of growth or alternative electro-acceptors are used by some community members. The stratification of the six-day StC and StB final-transfer communities might reflect a trade-off between fast-growth in the high-O_2 region and slower-growth in the low-O_2 region where competition may be reduced.

The finding that 28/32 soil-wash and six-day ShC, StC and StB strains (n=8 of each) form detectable biofilms whilst also occupying the low-O_2 region in static test microcosms suggest that physiological plasticity may play an important role in allowing the serial-transfer communities to maximise productivity by colonising both regions of static microcosms. Such plasticity is an attribute of generalists, and biofilm-formers may be selected despite the constant transfers, as generalists generally more successful under fluctuating conditions than constant conditions which selects for specialists (Van den Bergh *et al.* 2018). Interspecies interactions have been shown to be involved in the localisation of species within spatially-structured flow-cell biofilms which may reduce the selection of competitive phenotypes (Røder *et al*. 2019), and similar interactions as well as resource distributions and viscosity limiting the diffusion of cells, nutrients and biofilm matrix components are likely to also play roles in localising community members to the high and low-O_2 regions in our microcosm system.

Migration between the high and low-O_2 regions of static microcosms

Swimming motility is required for the efficient enrichment of cells to the high-O_2 region of static microcosms and in swimming motility assays, 29/32 soil-wash and six-day ShC, StC and StB strains

(*n*=8 of each) were found to be motile (1.6–5.2mm in 48h on soft-agar), suggesting that they could migrate from one region of the liquid column to another if required (random diffusion of non-motile cells takes too long in the context of microcosm colonization, Jerdan *et al.* 2019; e.g. SBW25 can swim at 77–100µm/s, Ping *et al*. 2013). Migration between these regions was demonstrated by transplant tests in which top and mid-column samples taken from static test microcosms inoculated with soil-wash, six-day ShC, StC and StB final-transfer community samples that were transferred to fresh static microcosms. In these biofilms subsequently formed and the liquid column was colonised in all cases (it should be noted that in these tests migration and growth are linked but as the random diffusion of cells is so slow, the presence of cells in both regions is evidence of migration). In these, transplant-origin did not significantly affect productivity (T-K HSD, α=0.05; **Supplementary Figure S9A**), but the biofilms produced from the StB biofilm-transplants were 3x stronger than those from the StB mid-column transplants (T-K HSD, α=0.05; **Supplementary Figure S9B**). This suggests that StB final-transfer community members are not equally distributed between the high and low-O_2 regions of static microcosm. In comparison, there was no significant difference in biofilm strengths between the six-day StC transplant microcosms (T-K HSD, α=0.05) which suggests that community members were more equally distributed and sampled during the StC serial-transfers. Migration in these microcosms is probably dominated by aerotaxis towards the A-L interface, but bacteria also respond to a wide range of chemical signals and micro-scale gradients within the biofilm may also affect bacterial motility and aggregation (Mitchell & Kogure, 2006).

Contribution of the high and low-O_2 regions to total productivity

Although community productivity in static test microcosms was the highest in the top 1 ml of the liquid column which included the A-L interface biofilm (**Figure 5**), 1.9–3.8x higher levels of productivity were found in the mid-column region (i.e. productivity summed across the following 2–5 ml samples down the liquid column; TK-HSD, α=0.05; **Figure 5E**). This demonstrates that the low-O_2 region dominates productivity in these community microcosms. However, the differences in productivities were lower for the six-day StB community than for the StC community, which may reflect the gradual loss of mid-column strains or migrants during the serial transfers of biofilm-only or mixed-community samples.

We explored further the relative contributions of the high and low-O_2 regions to total productivity by manipulating incubation conditions to change the oxygenation levels normally found in static microcosms. All final-transfer communities and the initial soil-wash inoculum were able to grow under low-O_2 conditions in our test microcosms suggesting that no region of our static microcosms could not be colonised by aerobic or facultative anaerobic strains. However, the serial-transfers had selected for faster-growing strains better suited to the conditions provided in these microcosms, as the productivities of the six-day ShC, StC and StB final-transfer communities were significantly greater than the initial soil-wash inoculum, and under higher-O_2 conditions StB but not StC community productivity was

significantly increased (TK–HSD, α=0.05; **Figure 6**). This also demonstrates that productivity is still O_2-limited in our microcosm model system (though with longer incubations the loss of nutrients and the build-up of toxic metabolites also becomes important). We noted that strain productivity was significantly higher under normal O_2 conditions compared to very-low O_2 conditions for soil-wash and six-day ShC, StC and StB strains (n=8 of each; Wilcoxon, P=0.01; see **Supplementary Figure S10** for community and strain responses to different oxygenation conditions). This suggests that these are all strict aerobes, but the growth of such strains may not be limited until O_2 levels are very low (Couvert *et al*. 2019) and some growth in the liquid column or in the biofilm below the top layer where O_2 levels remain high may still be possible in static microcosms (Koza *et al*, 2011; localised O_2 depletion may also occur within cell aggregates at the micro-scale, Wessel *et al*, 2014).

These changes in community productivity can be explained by a simple model, based on the perspective of resource competition within a spatially-structured environment and trade-offs between growth rate and metabolism (Bachmann *et al*. 2016). In this, microcosms are colonised by fast-growing aerobic strains that form biofilms and colonise the liquid column at low densities, and slower-growing micro-aerobic strains that might form biofilms under less-competitive conditions colonise the liquid column at higher densities. This model suggests that the serial-transfer of biofilm material will lead to the progressive loss of micro-aerobes and that the StB final-transfer communities should be dominated by aerobic strains. In contrast, the transfer of mixed-community samples should retain both biofilm-forming aerobic strains and micro-aerobes in the StC final-transfer communities.

Concluding comments

We have explored community-based A-L interface biofilm-formation and productivity in serial-transfer microcosms in which we expected O_2 availability and incubation periods to select for changes in community structure and trait characteristics. We have seen changes in community-aggregated traits associated with multispecies biofilm-formation measured at the community and individual-strain levels between the initial soil-wash inoculum and final-transfer communities. Although biofilm–formation was visually obvious in static microcosms, higher-levels of productivity were found in the O_2-depleted liquid column than in the high-O_2 region which includes these biofilms. The stratified nature of the final-transfer communities is maintained by strains demonstrating a degree of physiological plasticity and the ability to migrate between the high and low-O_2 regions of static microcosms.

This relatively simple model system illustrates the likely complexity of biofilm-forming communities which are found in a wide range of environments and contexts. It has also identified a linkage between the high-O_2 region and the low-O_2 liquid column of static microcosms. This suggests that studies of community-based (multispecies) biofilms should include the surrounding non-biofilm space to better understand the impact on biofilm–formation and persistence by migration of bacteria with plastic

phenotypes. In this context, bacterial migration might have serious consequences for the control and elimination of pathogens in medical, veterinary and agricultural contexts, and in the control and maintenance of communities used in food production and other industrial biomass-conversion processes.

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Supplementary information

Supplementary information text, data files and figures (S1–S10).

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References

Abriat C, Enriquez K, Virgilio N *et al*. Mechanical and microstructural insights of *Vibrio cholerae* and *Escherichia coli* dual-species biofilm at the air-liquid interface. *Colloids Surfaces B: Biointerfaces* 2020;**188:**110786.

Bachmann H, Bruggeman FJ, Molenaar D *et al*. Public goods and metabolic strategies. *Curr Opin Microbiol* 2016;**31:**109–115.

Bailey MJ, Thompson IP. Detection systems for phyllosphere pseudomonads. In: Wellington EMR and van Elsas JD (eds). *Genetic Interactions Between Microorganisms in the Natural Environment.* Oxford, Pergamon Press; 1992, 127–141.

436 Battin TJ, Sloan WT, Kjelleberg S *et al*. Microbial landscapes: new paths to biofilm research. *Nat Rev*
437 *Microbiol* 2007;**5:**76–81.

438 Brune A, Frenzel P, Cypionka H. Life at the oxic–anoxic interface: microbial activities and adaptations.
439 *FEMS Microbiol Rev* 2000;**24:**691–710.

440 Castledine M, Buckling A, Padfield D. A shared coevolutionary history does not alter the outcome of
441 coalescence in experimental populations of *Pseudomonas fluorescens. Evolutionary Biol*
442 2019;**32:**58–65.

443 Cavaliere M, Feng S, Soyer OS *et al.* Cooperation in microbial communities and their biotechnological
444 applications. *Environ Microbiol* 2017;19:2949–2963.

445 Cordero OX, Datta MS. Microbial interactions and community assembly at microscales. *Curr Opin*
446 *Microbiol* 2016;**31:**227–234.

447 Couvert O, Divanac'h M-L, Lochardet A *et al.* Modelling the effect of oxygen concentration on bacterial
448 growth rates. *Food Microbiol* 2019;**77:**21–25.

449 Downie HF, Valentine TA, Otten W *et al.* Transparent soil microcosm allow 3D spatial quantification of
450 soil microbiological processes *in vivo*. *Plant Signaling Behav* 2014;9:e970421

451 Estrela S, Libby E, Van Cleve *et al.* Environmentally mediated social dilemmas. *Trends Ecol Evol*
452 2019;**34:**6–18.

453 Fenchel T, Finlay B. Oxygen and the spatial structure of microbial communities. *Biol Rev* 2008;**83:**553–
454 569.

455 Fiegna F, Moreno-Letelier A, Bell T *et al.* Evolution of species interactions determines microbial
456 community productivity in new environments. *ISME J* 2015;**9:**1235–45.

457 Fierer N, Baberán A, Laughlin DC. Seeing the forest for the genes: using metagenomics to infer the
458 aggregated traits of microbial communities. *Frontiers Microbiol* 2014;**5:**Article 614.

459 Flemming H-C, Wingender J, Szewyk U *et al*. Biofilms: an emergent form of life. *Nat Rev Microbiol*
460 2016;**14:**563–757.

461 Hibbing ME, Fuqua C, Parsek MR *et al*. Bacterial competition: surviving and thriving in the microbial
462 jungle. *Nat Rev Microbiol* 2010;**8:**15–25.

463 Hill N, Pedley T. Bioconvection. *Fluid Dyn Res* 2005;**37:**1–20.

Hodapp D, Hillebrand H, Striebel M. “Unifying” the concept of resource use efficiency in ecology. Front Ecol Evol 2019;**6:**233.

Howard MM, Bell TH, Kao-Kniffin J. Soil microbiome transfer method affects microbiome composition, including dominant microorganisms, in a novel environment. *FEMS Microbiol Lett* 2017;**364:**fnx092.

Humphris SN, Bengough AG, Griffiths BS *et al.* Root cap influences root colonisation by *Pseudomonas fluorescens* SBW25 on maize. *FEMS Microbiol Ecol* 2005;**54:**123–130.

Jerdan R, Kuśmierska A, Petric M *et al.* Penetrating the air–liquid interface is the key to colonization and wrinkly spreader fitness. *Microbiology* 2019;**165:**1061–74.

Kaltz O, Escobar-Páramo P, Hochbery ME et al. Bacterial microcosms obey Taylor’s law: effects of abiotic and biotic stress and genetics on mean and variance of population density. *Ecological Processes* 2012;**1:**5.

Koza A, Kuśmierska A, McLaughlin K *et al*. Adaptive radiation of *P. fluorescens* SBW25 in experimental microcosms provides an understanding of the evolutionary ecology and molecular biology of A-L interface biofilm-formation. *FEMS Microbiology Letters* 2017;**364:**fnx109.

Koza A, Moshynets O, Otten W *et al.* Environmental modification and niche construction: developing O_2 gradients drive the evolution of the Wrinkly Spreader. *ISME J* 2011;**5:**665–73.

Kuśmierska A, Spiers AJ. New insights into the effects of several environmental parameters on the relative fitness of a numerically dominant class of evolved niche specialist. *Int J Evolutionary Biol* 2016; Article ID 4846565, 10 pages.

Lawrence D, Fiegna F, Behrends V *et al.* Species interactions alter evolutionary responses to a novel environment. *PLoS Biology* 2012;**10:**e1001330.

Liu J, Meng Z, Liu X, Zhang X-H. Microbial assembly, interaction, functioning, activity and diversification: a review derived from community compositional data. *Marine Life Sci Technol* 2019;**1:**112–28.

Loudon CM, Mattews B, Sevilgen DS *et al*. Experimental evidence that evolution by niche construction affects dissipative ecosystem dynamics. *Evol Ecol* 2016;**30:**221–34.

Mitchell JG, Kogure K. Bacterial motility: links to the environment and a driving force for microbial physics. *FEMS Microbiol Ecol* 2006;**55:**3–16.

493 Moshynets OV, Spiers AJ. Viewing biofilms within the larger context of bacterial aggregations. In:
494 *Microbial biofilms – Importance and applications.* Dhanasekaran D & Thajuddin N (Eds.). Rijeka:
495 InTech Publishers, 2016, p. 3-22.

496 Nadell CD, Drescher K, Foster KR. Spatial structure, cooperation and competition in biofilms. *Nat Rev*
497 *Microbiol* 2016;**14:**589–600.

498 Nadell CD, Xavier JB, Foster KR. The sociobiology of biofilms. *FEMS Microbiol Rev* 2009;**33:**206–
499 224.

500 Ping L, Birkenbeil J, Monajembashi S. Swimming behaviour of the monotrichous bacterium
501 *Pseudomonas fluorescens* SBW25. *FEMS Microbiol Ecol.* 2013;**86:**36–44.

502 Rainey PB, Bailey MJ. Physical and genetic map of the *Pseudomonas fluorescens* SBW25 chromosome.
503 *Mol Microbiol* 1996;**19:**521–33.

504 Robertson M, Hapca SM, Moshynets O *et al.* Air-liquid interface biofilm formation by psychrotrophic
505 pseudomonads recovered from spoilt meat. *Antonie van Leeuwenhoek* 2013;**103:**251–9.

506 Røder HL, Liu W, Sørensen SJ *et al.* Interspecies interactions reduce selection for a biofilm- optimized
507 variant in a four-species biofilm model. *Environmental Microbiol Reports* 2019;**11:**835–839.

508 Schleck D, Barraud N, Klebensberger J *et al*. *Pseudomonas aeruginosa* PAO1 preferentially grows as
509 aggregates in liquid batch cultures and disperses upon starvation. *PLoS ONE* 2009;**4:**e5513.

510 Smith VH. Microbial diversity-productivity relationships in aquatic ecosystems. *FEMS Microbiol Ecol*
511 2007;**62:**181–186.

512 Tan CH, Lee KWK, Burmølle M et al. All together now: experimental multispecies biofilm model
513 systems. *Environ Microbiol* 2017;**19:**42–53.

514 Tyc O, de Jagger VCL, van den Berg M, *et al.* Exploring bacterial interspecific interactions for
515 discovery of novel antimicrobial compounds. *Microbial Biotechnol* 2017;10:910–925.

516 Travisano M. The effects of toxic metabolites on dynamics and fitness in laboratory populations. In:
517 *Microbial Diversity and Biodegradation.* Horikoshi K, Fukuda M, Kudo T (Eds.). Tokyo: Japan
518 Sci Societies Press, 1997, p. 97 -112.

519 Ude S, Arnold DL, Moon CD *et al*. Biofilm formation and cellulose expression among diverse
520 environmental *Pseudomonas* isolates. *Environ Microbiol* 2006;**8:**1997-2011.

Van den Bergh B, Swings T, Fauvart M *et al.* Experimental design, population dynamics, and diversity in microbial experimental evolution. *Microbiol Mol Biol Rev* 2018;**82:**e00008-18.

Wellborn GA, Langerhans RB. Ecological opportunity and the adaptive diversification of lineages. *Ecol Evolut* 2015;**5:**176-195.

Wessel AK, Arshad TA, Fitzpatrick M *et al.* Oxygen limitation within a bacterial aggregate. *mBio* 2014;**5:**e00992-14.

Zhang Q-G, Ellis RJ, Godfray HCJ. The effect of a competitor on a model adaptive radiation. *Evolut* 2012;**66:**1985-1990.

Figure Legends

Figure 1 **Community productivity changes across treatments in the serial-transfer experiment.** Productivity (OD_{600}) data from the first and last incubations of the serial-transfer experiment were analysed to determine whether changes had occurred across treatments. Shown here are community replicate and pooled community data for the shaken incubation, mixed-community sample transfers (ShC, Panel A), static incubation, mixed-community sample transfers (StC, Panel B), and static incubation, biofilm-only sample transfers (StB, Panel C) for each of the first and final incubations and for the one, three and six-day incubation periods. The median (small black circles) of the microcosm replicates (n=7, small white circles) is shown for each of the three community replicates. Trend-lines between community medians (larger grey boxes with ranges indicated, pooled data, n=21) are shown linking one, three and six-day incubations. A mixed-effects modelling approach was used to investigate treatments (see the main text and Supplementary Information for more details).

Figure 2 **Community-aggregated traits measured at the community-level change across treatments in the serial-transfer experiment.** The combined biofilm assay was used to measure productivity, biofilm strength and attachment levels in the serial-transfer experiment and Principal components analysis of these data used to visualise changes in community-aggregated traits measured at the community-level between treatments (squares, ShC (shaken incubation with mixed community sample transfers); diamonds, StC (static incubation with mixed-community sample transfers); circles, StB (static incubation with biofilm-only sample transfers) final-transfer communities). The same plot has been reproduced to high-light changes according to one, three and six-day incubation periods (Panels A–C) and ShC, StC and StB incubation conditions and sample transfer types (Panels D–F), with the shift from the first to final-transfer microcosms indicated by arrows. Shown are the means of community replicates (n=3). The two principal components shown here account for 71.9% of the variation and the Eigenvectors (A, attachment; P, productivity; S, strength) are indicated in Panel F.

Figure 3 **Community-aggregated traits measured at the individual strain-level change across treatments in the serial-transfer experiment.** The combined biofilm assay was used to measure productivity, biofilm strength and attachment levels of individual strains isolated from the initial soil-wash inoculum (white circles) and final-transfer communities (black circles) (n=24 from each) in static test microcosms, and Principal components analysis of these data used to visualise changes in community-aggregated traits at the strain-level between treatments. The same plot has been reproduced to high-light differences between

strains recovered from the soil-wash (SW) inoculum and final-transfer communities (Panels A–F; one, three and six-day ShC (shaken incubation with mixed community sample transfers), StC (static incubation with mixed-community sample transfers) and StB (static incubation with biofilm-only sample transfers) final-transfer communities) with strain clustering suggested by ovals. Means (n=8) were used for this analysis, and the two principal components shown here account for 80.5% of the variation and the Eigenvectors (A, attachment; P, productivity; S, strength) are indicated in Panel I.

Figure 4 **Productivity-richness relationships for simple strain-mixtures and communities.** The productivity (OD_{600}) of single strains and simple mixtures of two, four or eight strains were compared to final-transfer StB (static incubation with biofilm-only sample transfer) community samples in static test microcosms (Panels A–C; strains and community samples from one, three and six-day StB final-transfer communities). The median (small white circles) of the microcosm replicates (n=8) is shown for each of the one, two and four-strain mixtures. The grey box with ranges indicated show the median of the eight-strain mixture and the community sample (each with n=8 replicates). Trend-lines between medians are shown linking one, two, four and eight-strain mixtures and the community samples. Significant differences from the community sample are indicated (Steel, P values).

Figure 5 **Final-transfer communities are stratified and the low-O_2 region is more productive than the high-O_2 region of static microcosms.** Static microcosms inoculated with the soil-wash inoculum and the six-day final-transfer community samples and incubated for 24 h in static test microcosms show differences in cell distribution (relative OD_{600}) down through the liquid column (Panels A–D; SW (soil-wash inoculum) and six-day ShC (shaken incubation with mixed community sample transfer), StC (static incubation with mixed-community sample transfer) and StB (static incubation with biofilm-only sample transfer) final-transfer community samples). This data can also be used to assess productivity which is higher mid-column in the low-O_2 region (grey circles) than in the high-O_2 region (black circles) at the top of the liquid column (Panel E). Six samples were taken sequentially from the top of the liquid column which includes the A-L interface biofilm, down to the bottom which includes the remains of the cell pellet used to inoculate the microcosm. Data are shown here as means with standard errors of the mean (n=5) but for clarity the error bars are not shown where they are smaller than the mean symbol. Mid-column productivity was calculated by summing productivity in Samples 2–5. Means not linked by the same letter within panels are significantly different (TK-HSD, α=0.05; trends are suggested by dashed lines).

Figure 6 **Increased oxygenation results in higher community productivities.** Productivity (change in OD_{600}) of the soil-wash inoculum and six-day final-transfer community samples was affected by oxygen availability in test microcosms which were incubated statically under very-low O_2 conditions, statically under normal conditions, or with shaking to provide high levels of aeration (Panels A–D; SW (soil-wash inoculum) and six-day ShC (shaken incubation with mixed community sample transfer), StC (static incubation with mixed-community sample transfer) and StB (static incubation with biofilm-only sample transfer) final-transfer community samples). Data are shown here as means with standard errors of the mean (n=3) but for clarity the error bars are not shown where they are smaller than the mean symbol. Means not linked by the same letter within panels are significantly different (TK-HSD, α=0.05; trends are suggested by dashed lines).

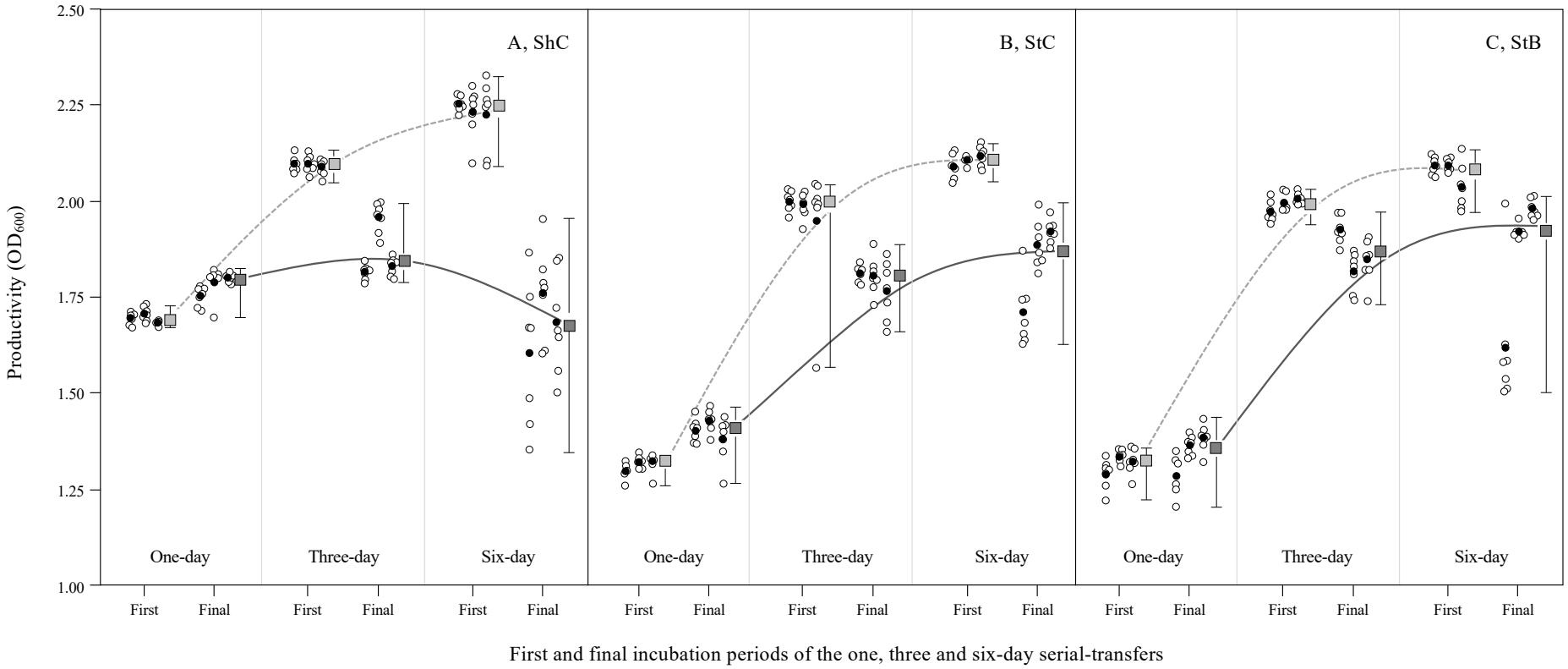

A, ShC
B, StC
C, StB
Productivity (OD600)
2.50
2.25
2.00
1.75
1.50
1.25
1.00
One-day
Three-day
Six-day
First
Final
First and final incubation periods of the one, three and six-day serial-transfers

Figure 1

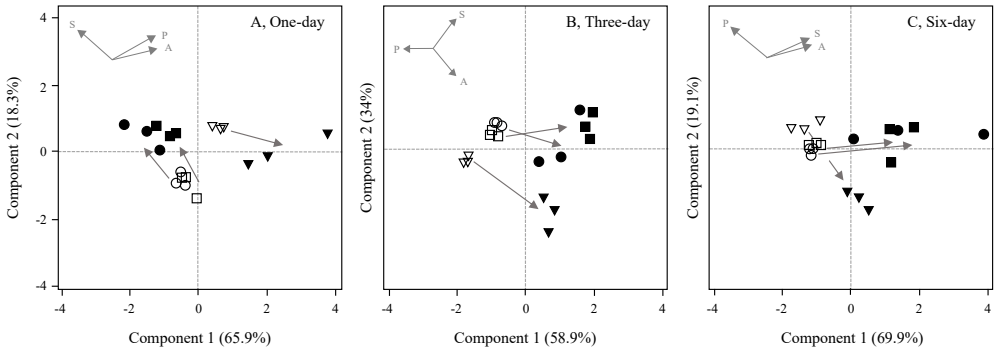

A, One-day
B, Three-day
C, Six-day
S
P
A
Component 2 (18.3%)
Component 2 (34%)
Component 2 (19.1%)
Component 1 (65.9%)
Component 1 (58.9%)
Component 1 (69.9%)
4
2
0
-2
-4

Figure 2

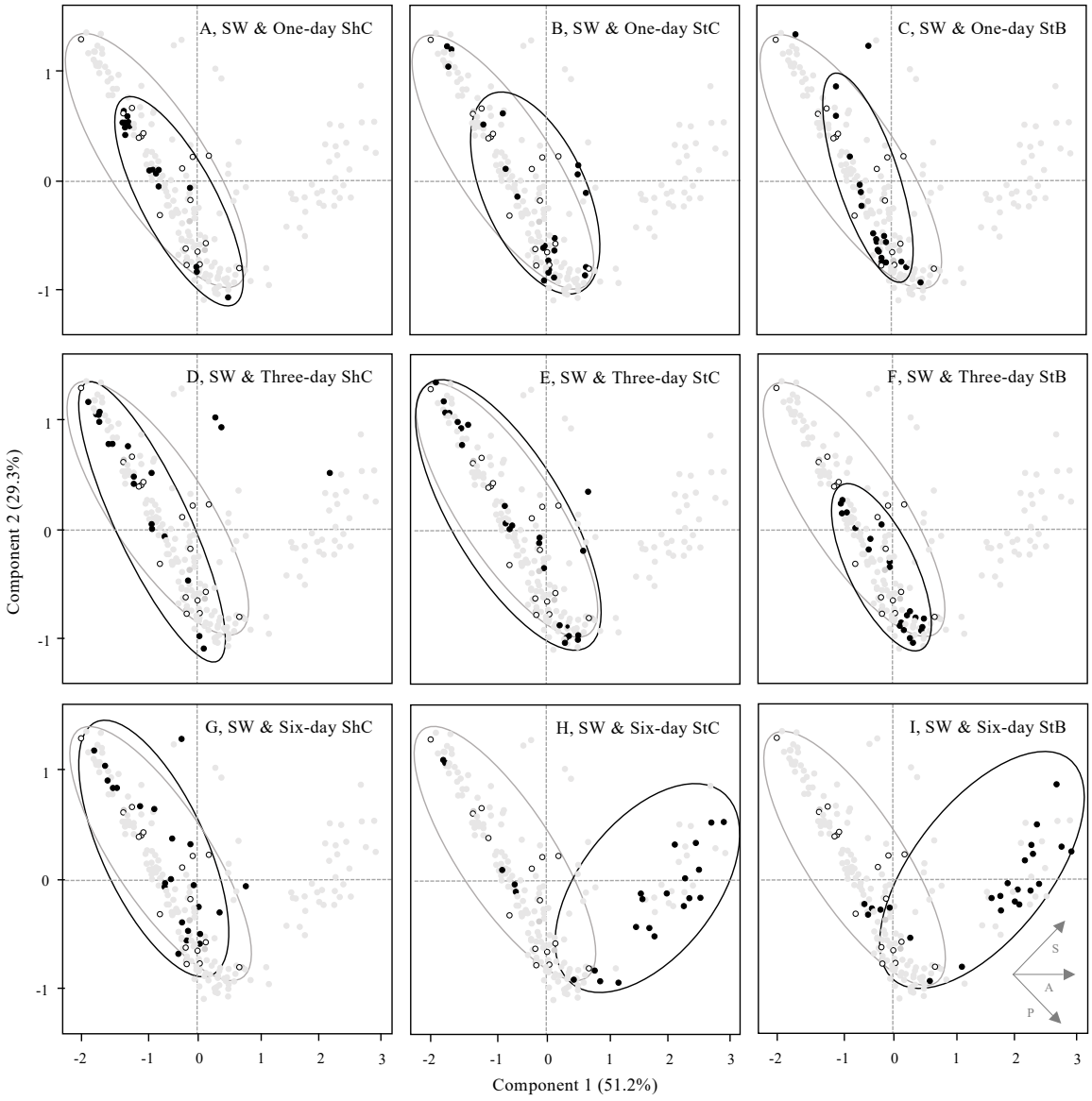

A, SW & One-day ShC
B, SW & One-day StC
C, SW & One-day StB
D, SW & Three-day ShC
E, SW & Three-day StC
F, SW & Three-day StB
G, SW & Six-day ShC
H, SW & Six-day StC
I, SW & Six-day StB
S
A
P
Component 2 (29.3%)
Component 1 (51.2%)

Figure 3

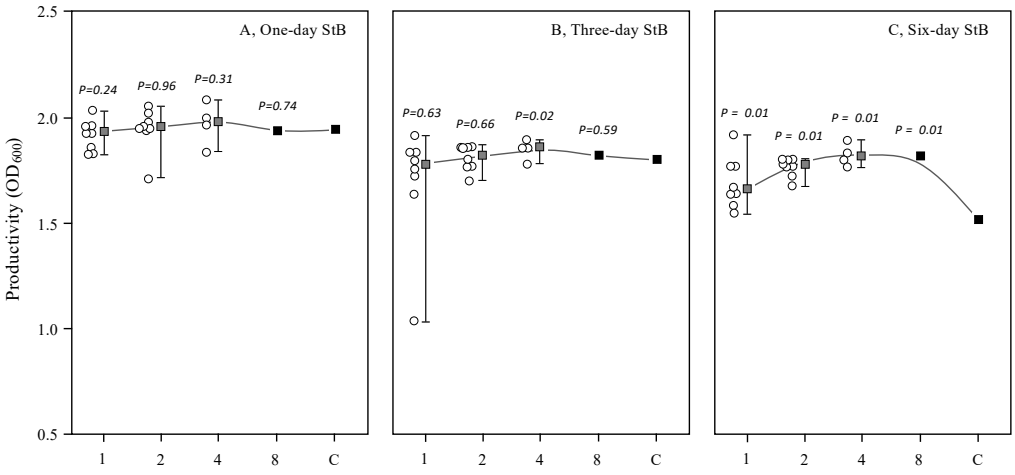

A, One-day StB
B, Three-day StB
C, Six-day StB
P=0.24
P=0.96
P=0.31
P=0.74
P=0.63
P=0.66
P=0.02
P=0.59
P = 0.01
P = 0.01
P = 0.01
P = 0.01
Productivity (OD600)
2.5
2.0
1.5
1.0
0.5
1
2
4
8
C
Number of strains (1 – 8 strains; c, community sample)

Figure 4

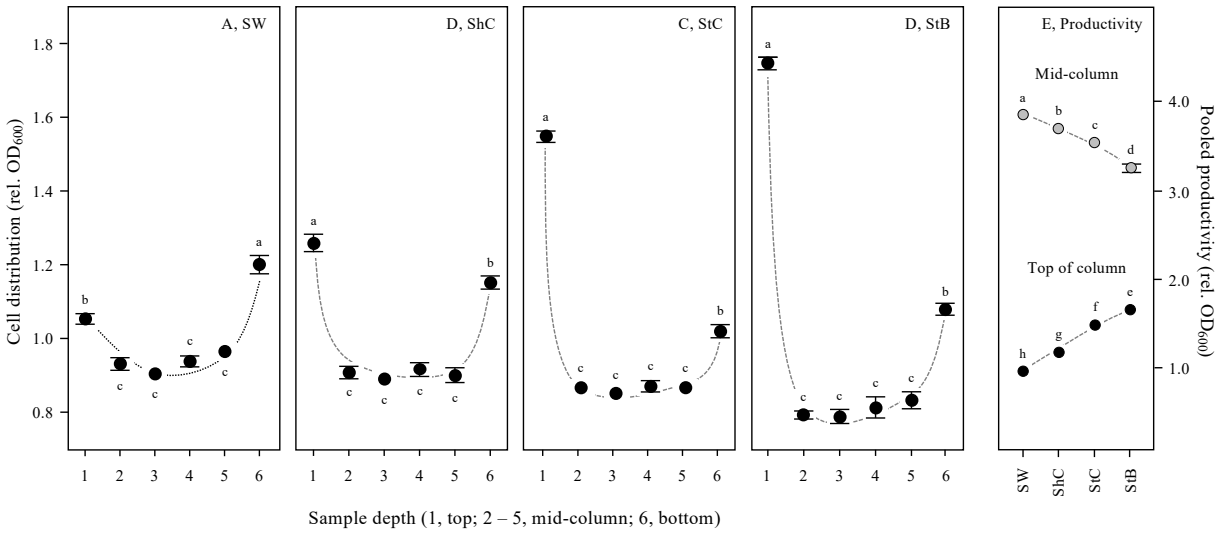

A, SW
D, ShC
C, StC
D, StB
E, Productivity
Mid-column
Top of column
Cell distribution (rel. OD600)
Pooled productivity (rel. OD600)
Sample depth (1, top; 2 – 5, mid-column; 6, bottom)
SW
ShC
StC
StB

Figure 5

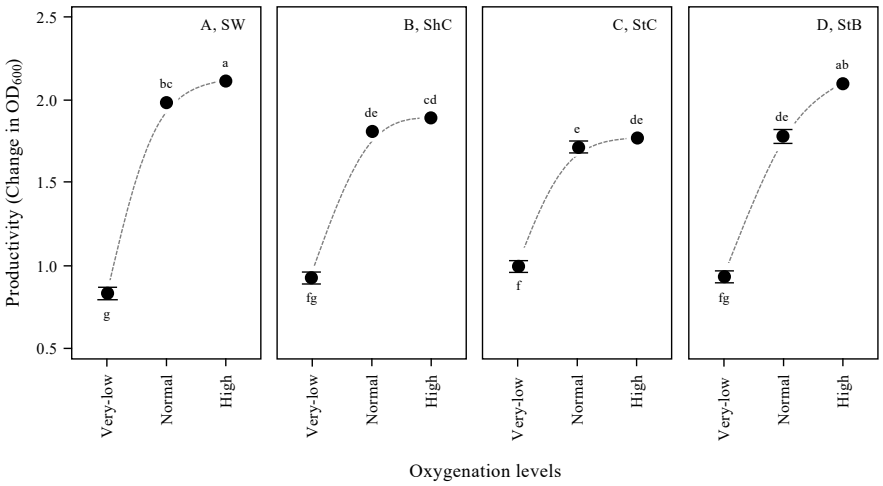

A, SW
B, ShC
C, StC
D, StB
Productivity (Change in OD600)
2.5
2.0
1.5
1.0
0.5
a
bc
g
de
cd
fg
e
de
f
ab
de
fg
Very-low
Normal
High
Oxygenation levels

Figure 6

Research Letter for FEMS Microbiology Letters

Community biofilm-formation, stratification and productivity in serially-transferred microcosms

Robyn Jerdan, Scott Cameron, Emily Donaldson, Olga Iungin, Olena V. Moshynets & Andrew J. Spiers

Supplementary Information

All references used here are listed in the main paper.

Strain isolation

Strains were isolated from soil-wash inocula and final-transfer community samples using a stratified random sampling approach. Independent dilution series were prepared and aliquots were spread on KB* plates and incubated for 2-3 days. A single plate in each serial dilution with clearly separated colonies was chosen, and from this, four colonies representing the most abundant colony morphologies (size, texture and colour) were selected. If fewer than four colony morphologies were found on the plate, then additional colonies were selected to reflect the dominant types. In total, 24 soil-wash strains and 24 strains from each of the final-transfer community treatments were recovered as stored at -80°C as glycerol stocks.

Biofilm and microcosm-based assays

The combined biofilm assay (Robertson *et al.* 2013) was used to measure productivity (OD_{600}), biofilm-strength (assessed using small glass balls, grams) and attachment levels (Crystal violet staining, A_{570}) in replicate microcosms (n=8) inoculated with community samples, single or mixed strains and incubated statically for three days. Cell distributions through the liquid column were assessed using replicate microcosms (n=5) and OD_{600} measurements from six 1ml samples taken from the top to the bottom of static microcosms (Jerdan *et al*. 2019), with relative OD_{600} calculated as the ratio of the OD_{600} / mean OD_{600}. Transplant tests used biofilm samples collected with a wire loop and 100µl samples taken mid-column from static microcosms, which were then transferred to fresh replicate microcosms (n=5) and incubated statically for three days before determining productivity and biofilm-strengths. Productivity under different oxygenation conditions was determined by OD_{600} measurements before and after 24h incubation of replicate shaken, static and very-low O_2 microcosms (n=3) and calculated as the change in

OD_{600}. The effect of aged culture media on growth (OD_{600}) was assessed using mixed and diluted-KB* media in shaken replicate microcosms (n=5) inoculated with *P. fluorescens* SBW25 and final-transfer community samples after 24h.

Strain phenotypes, pair-wise interactions, community richness and diversity indices

Strain phenotypes were assessed by gram staining, growth on Oxoid PSA-CFC plates (Thermo Scientific, UK), culture acidity, catalase and oxidase activity, swimming motility, siderophore and surfactant production (Robertson *et al*. 2013). Pair-wise interactions were investigated by spreading 'test' strains on KB* plates and then adding 10µl aliquots of 'spot' strains and incubating for two days before inspection for reduced growth or growth-exclusion, suggesting competitive or antagonistic interactions, respectively. Categorical strain phenotype data, as well as individual combined biofilm assay strain data converted into three-digit codes based on the quartile distributions of each measurement were used to determine community richness and Shannon and Inverse Simpson's diversity indices.

Establishing the serial-transfer community microcosm system

We designed a serial-transfer experiment using soil-wash inocula and liquid microcosms to investigate how communities respond to restricted growth periods and limiting resources and maximise productivity. Soil-wash inocula provide a mixed assemblage of colonists, which in our case has been enriched for fast-growing aerobic pseudomonads likely to be biofilm-competent (Ude *et al.* 2006), and although they generally provide lower-diversity than the original soil aggregate samples themselves (Howard *et al*. 2017), aliquots stored at -80°C provide standardised inocula for experiments when required.

Our experimental system was based on preliminary tests using high-nutrient (30 mg Carbon ml^{-1}) KB* microcosms which demonstrated that incubation period and static and shaken-incubation had a significant effect on community productivity (Mixed-effects model with microcosm replicate as a random effect; A-D, $P = 0.42$; *RSquare* = 0.89) where *microcosm* ($n = 8$) contributed 0.0% to the total variance components and incubation period (1, 3 & 6 days; $F_{2,2} = 203$; $P = 0.01$) and conditions (static & shaken; $F_{1,1} = 31$, $P = 0.01$) were included as fixed effects). In these, productivity was significantly higher for longer incubations (1 day < 3 day < 6 day; LSMeans Differences Tukey HSD, $Q = 2.5$, $\alpha = 0.05$) and in shaken microcosms (static < shaken; LSMeans Differences Student's t, $\alpha = 0.05$) (productivity could also be supressed using antibiotics, heavy metals and perchlorate, which could provide other opportunities in this system; data not shown). This suggested that productivity was limited by time and O_2 availability rather than by nutrients in our system, and importantly, the difference between potential and realized productivity (i.e. longer/shaken *cf.* short/static incubations) reflects an opportunity for community change to maximise productivity during serial-transfers, as resource use efficiency can constrain productivity (Hodapp *et al.* 2019). The transfer of relatively large samples would

avoid any bottleneck-effect which might otherwise lead to community collapse or domination of the community by a relatively few strains.

We explored this further by transferring communities coalescing from soil-wash inocula ten-times across eleven microcosms over a total of 30 – 60 days, which we calculated would involve ~60 'community' generations (i.e. the average number of generations across all strains) based on the number of doublings required to reach carrying capacity after each transfer. This should be sufficient to see differences between the initial soil-wash inoculum and final-transfer communities, as evolutionary and ecological processes have been observed over relatively short evolutionary time-scales of 40–70 bacterial generations in other serial-transfer experiments (e.g. Kaltz *et al.* 2012; Lawrence *et al.* 2012; Fienga *et al.* 2015; Castledine *et al.* 2019). Inspection of KB* plates spread with soil-wash inocula and final-transfer samplers confirmed community diversity in terms of strain variation in colony morphology (size, texture and colour) but did not find any indications of bacteriophage (plaquing) or fungi, or evidence of protists or nematodes (i.e. trails on the agar surface or through colonies, or worms) which might have been in the original soil samples.

In addition, while productivity and other measurements can be made during the serial-transfer experiment itself, further assays can be undertaken in standardised three-day static 'test' microcosms inoculated with either samples from the final-transfer communities or strains to better understand the changes that have occurred in the serial-transfer experiment. In particular, soil-wash inoculum and shaken-incubation final-transfer communities and strains would be expected to be poorly adapted to these conditions, as the inoculum has not undergone a substantive period of community coalescence and the shaken-incubation community had been selected under conditions where biofilms cannot form.

Analysis of productivity from the serial-transfer experiment

We chose to use a linear mixed-effects modelling approach using random and fixed effects to investigate the effect of serial-transfer experimental treatments on productivity (the complete serial-transfer data set is provided in an accompanying Excel spreadsheet). Microcosm replicates (n = 7) were nested within community replicates (n = 3) to avoid potential pseudo-replication issues, and both microcosm and community replicates were random effects. We chose not to remove outliers, as most were associated with differences between community replicates and we had no reason to remove one replicate rather than another, and as a result accepted some non-Normality in residuals (but see our subsequent confirmation of models after outlier analyses). We did not allow singularities or other loss of degrees of freedom and required high Summary of Fit (*RSquare*) values to produce robust models.

Mixed-effects model of productivity using the first and last serial-transfer incubations

In order to determine whether there had been significant changes across the serial-transfer experiment, we modelled productivity (OD_{600}) for the first and last incubations as the response for a mixed-effects

model (**Model 1 (a)**; *RSquare* = 0.90) in which *microcosm[community]* and *community* contributed 0.0 and 7.2% to the total variance components. Incubation *period* (1, 3 & 6 days; $F_{2,2}$ = 628, P = 0.01) and *conditions* (static & shaken; $F_{1,1}$ = 188, P = 0.01), *sample type* (mixed-community & biofilm; $F_{1,1}$ = 0.1; P = 0.91) and *shift* (first & last incubations; $F_{1,1}$ = 285, P = 0.01) were included as fixed effects, and *period x conditions* ($F_{2,2}$ = 110, P = 0.01), *period x sample type* ($F_{2,2}$ = 3.5, P = 0.03) and *period x shift* ($F_{2,2}$ = 179, P = 0.01) were included as interactions. Significant differences in productivity were found within effects and interactions (LSMeans Differences Student's *t* and Tukey HSD; α = 0.05) including *period* (1 day < 3 & 6 day; Q = 2.4), *conditions* (static < shaken), *shift* (last < first), *period x conditions* (1 day/static < 1 day/shaken < 3 day/shaken < 6 day/shaken, 6 day/static & 3 day/shaken; Q = 2.9), *period x sample type* (1 day/biofilm & 1 day/mixed-community < 3 day/mixed-community, 6 day/biofilm, 6 day/mixed-community & 3 day/biofilm; Q = 2.8). This analysis clearly demonstrates that significant changes in productivity had occurred between the first and last incubations of the serial-transfer experiment across treatments, except mixed community and biofilm-only sample types.

In order to confirm the model outcomes, we decided to check whether residual non-Normality biased the effects and interaction tests. In order to do this, we systematically re-ran the same model and removed outliers identified by an inspection of residuals and testing the goodness-of-fit of a Normal distribution using the Anderson-Darling (A-D) test. In total, eleven iterations of modelling and inspection were conducted, reducing Observations (Sum Weights) from 377 to 299 without singularities or other losses of degrees of freedom, before Normality was obtained (A-D, P = 0.11). In this mixed-effects model (**Model 1 (b)**; *RSquare* = 0.99) *microcosm[community]* and *community* contributed 1.9 and 7.8% to the total variance components. All effects were significant (*period*, $F_{2,2}$ = 4816; *conditions*, $F_{1,1}$ = 2008; *sample type*, $F_{1,1}$ = 269; *shift*, $F_{1,1}$ = 823; P = 0.01). *Period x conditions* ($F_{2,2}$ = 558, P = 0.01) and *period x shift* ($F_{2,2}$ = 779, P = 0.01) interactions were significant (P = 0.01) but not *period x sample type* ($F_{2,2}$ = 1.4, P = 0.24). Significant differences in productivity were found within effects and interactions (LSMeans Differences Student's *t* and Tukey HSD; α = 0.05) including *period* (1 day < 3 day < 6 day; Q = 2.4), *conditions* (static < shaken;), *shift* (last < first), *period x conditions* (1 day/static < 1 day/shaken < 3 day/static < 3 day/shaken < 6 day/static < 6 day/shaken; Q = 2.9), *period x sample type* (1 day/biofilm < 1 day/mixed-community < 3 day/biofilm & 3 day/mixed-community < 6 day/biofilm & 6 day/mixed-community; Q = 2.9) and *period x shift* (1 day/first < 1 day/last < 3 day/last < 6 day/last < 3 day/first < 6 day/first; Q = 2.9). This mixed-effects model was in agreement with **Model 1 (a)** and confirms model outcomes, but clearly has more discriminating power that shows that sample type was a significant effect.

Mixed-effects model of productivity using all serial-transfer incubations

We decided to see whether the effects and interactions seen in the first and last incubations mixed-effects model were also apparent in an analysis of productivity (OD_{600}) from all of the serial-transfer incubations

(n = 11). In this mixed-effects model (**Model 2**; *RSquare* = 0.78) *microcosm[community]* and *community* contributed 0.0 and 0.1% to the total variance components. Incubation *period* (1, 3 & 6 days; $F_{2,2}$ = 2384, P = 0.01) and *conditions* (static & shaken; $F_{1,1}$ = 1312, P = 0.01), *sample type* (mixed-community & biofilm; $F_{1,1}$ = 2.0; P = 0.14), and *transfer number* (microcosms 1 – 11; $F_{10,10}$ = 48, P = 0.01) were included as fixed effects, and *period x transfer* ($F_{20,20}$ = 25, P = 0.01) and *period x sample type* ($F_{2,2}$ = 303, P = 0.01) were included as interactions. Significant differences in productivity were found within effects (LSMeans Differences Student's t and Tukey HSD; α = 0.05) including *period* (1 < 3 < 6 days; Q = 2.4), *condition* (static < shaken) and *period x sample type* (1 day/biofilm < 1 day/mixed-community < 3 & 10 day/mixed-communities < 3 day/biofilm < 6 day/biofilm; Q = 2.9) (LSMeans Differences Student's t and Tukey HSD; α = 0.05). This analysis of productivity across all incubations is broadly in agreement with **Model 1 (a)**. However, we could not confirm the model outcomes after outlier analysis, as we were unable to achieve Normality before the loss of degrees of freedom occurred.

Analysis of productivity by including biofilm strength and attachment data

We were interested in determining whether productivity was affected by biofilm strength or attachment, as biofilms could allow substantially more bacteria to remain in the high-O_2 region of static microcosms and consequently have a greater impact on microcosm productivity. Productivity (OD_{600}) from the serial-transfer experiment was analysed as before using a mixed-effects model for the first and final incubations (**Model 3 (a)**; *RSquare* = 0.81) where *microcosm[community]* and *community* contributed 0.0 and 5.7% to the total variance components. Biofilm *attachment* (A_{570}, $F_{1,1}$ = 9.2, P = 0.01) and *strength* (g, $F_{1,1}$ = 5.3, P = 0.02), incubation *period* (1, 3 & 6 days; $F_{2,2}$ = 34, P = 0.01) and *conditions* (static & shaken; $F_{1,1}$ = 73, P = 0.01), *sample type* (mixed-community & biofilm; $F_{1,1}$ = 0.01; P = 0.98), and *shift* (between first and last transfers, $F_{1,1}$ = 57, P = 0.01) were included as fixed effects, and *period x attachment* ($F_{2,2}$ = 7.3, P = 0.01), *period x strength* ($F_{2,2}$ = 4.7, P = 0.01), *period x conditions* ($F_{2,2}$ = 56, P = 0.01), *period x sample type* ($F_{2,2}$ = 2.1, P = 0.13) and *attachment x strength* ($F_{1,1}$ = 0.04, P = 0.84) included as interactions. This suggests that biofilms contribute significantly to microcosm productivity and that more substantial biofilms are produced with longer incubation periods.

In order to confirm the model outcomes, we re-ran the model after outlier analysis and achieving Normality of residuals with a reduction of Observations (Sum Weights) from 378 to 293 (A-D, P = 0.08). In this mixed-effects model (**Model 3 (b)**, *RSquare* = 0.99) where *microcosm[community]* and *community* contributed 0.0 and 1.2% to the total variance components. Biofilm *attachment* (A_{570}, $F_{1,1}$ = 11.6, P = 0.01) and *strength* (g, $F_{1,1}$ = 7.7, P = 0.01), incubation *period* (1, 3 & 6 days; $F_{2,2}$ = 1058, P = 0.01) and *conditions* (static & shaken; $F_{1,1}$ = 1715, P = 0.01), *sample type* (mixed-community & biofilm; $F_{1,1}$ = 5.2; P = 0.02), and *shift* (between first and last transfers, $F_{1,1}$ = 22, P = 0.01) were included as fixed effects, and *period x attachment* ($F_{2,2}$ = 0.05, P = 0.95), *period x strength* ($F_{2,2}$ = 3.9, P = 0.02), *period x conditions* ($F_{2,2}$ = 322, P = 0.01), *period x sample type* ($F_{2,2}$ = 3.1, P = 0.05), *period x shift* ($F_{2,2}$ = 96, P =

0.01) and *attachment x strength* ($F_{1,1} = 15$, $P = 0.01$) included as interactions. This mixed-effects model was in agreement with **Model 3 (a)** and confirms model outcomes, but clearly has more discriminating power that shows that sample type was a significant effect.

Analysis of individual strain productivity

In order to determine whether strain productivity varied depending on the soil wash or final-transfer community they were isolated from, productivity (OD_{600}) was analysed using a mixed-effects model (**Model 4 (a)**; *RSquare* = 0.96) where *microcosm* ($n = 8$) contributed 0.6% to the total variance components (the complete CBA data set is provided in an accompanying Excel spreadsheet). Biofilm *attachment* (A_{570}, $F_{1,1} = 20$, $P = 0.01$) and *strength* (g, $F_{1,1} = 0.4$, $P = 0.51$), *strain origin* (Soil wash, 1-D ShC, 1-D StC, 1-D StB, 3-D ShC, 3-D StC, 3-D StB, 6-D ShC, 6-D StC & 6-D StB, $F_{9,9} = 87$, $P = 0.00$), *strain* (240 strains) nested in *origin* ($F_{231,231} = 98$, $P = 0.01$) were included as fixed effects, and *attachment x origin* ($F_{9,9} = 15$, $P = 0.01$), *strength x origin* ($F_{9,9} = 1.44$, $P = 0.19$) and *attachment x strength* ($F_{1,1} = 22$, $P = 0.01$) included as interactions. Significant differences in productivity were found within effects (LSMeans Differences Tukey HSD; $\alpha = 0.05$) including *strain[origin]* (too many to list, but no strain was uniquely differentiated; $Q = 4.7$) and *origin* (1-D ShC, 3-D ShC, 3-D StC & 6-D ShC < Soil wash, 1-D StB, 1-D StC, 6-D StB, 6-D StC & 3-D StB; $Q = 3.2$). This demonstrates that strain productivity varies considerably and reflects their origin and suggests that the serial-transfer experiment had also differentiated communities at the individual strain level.

In order to confirm the model outcomes, we undertook outlier analysis but were unable to achieve Normality before the loss of degrees of freedom occurred. We therefore simplified the mixed-effects model to include only *strain[strain origin]* and *origin* and achieved Normality when Observations (Sum Weights) were reduced from 1919 to 728 (A-D, $P = 0.08$). In this mixed-effects model (**Model 4 (b)**; *RSquare* = 0.99) *microcosm* contributed 1.6% to the total variance components. *Strain[strain origin]* ($F_{216,216} = 18231$) and *strain origin* (Soil wash, 1-D ShC, 1-D StC, 1-D StB, 3-D ShC, 3-D StC, 3-D StB, 6-D ShC, 6-D StC & 6-D StB; $F_{9,9} = 96121$) were significant effects ($P = 0.01$). This model was able to differentiate between all strain origins (LSMeans Differences Tukey HSD; $Q = 3.2$, $\alpha = 0.05$. This mixed-effects model was in agreement with **Model 4 (a)** and confirms model outcomes.

Finally, across all strains, positive correlations were found between productivity and strength ($\rho = 0.32$, $P = 0.01$), productivity and attachment ($\rho = 0.38$, $P = 0.01$), and strength and attachment ($\rho = 0.30$, $P = 0.01$) (correlations were similar for strains recovered from static final-transfer treatments, $\rho = 0.19$, 0.26, 0.39, $P = 0.01$). This suggests that biofilms produced by individual strains were contributing to microcosm productivity (i.e. through increased biomass localised to the high O_2 region) and that strong biofilms were also well-attached structures.

Supplementary Figure Legends

Figure S1. Replicate communities, treatments and transfer sample types. In our serial-transfer experiment, replicate communities (arrows) were established using soil-wash (SW) inoculum (grey circles) and samples transferred ten times across a total of eleven microcosms with nine treatments (Panel A). Microcosms were vigorously mixed before sampling for mixed-community transfer) or the biofilms directly sampled using a wire loop for the biofilm-only transfer (Panel B). The treatments were one, three and six-day ShC (shaken incubations with mixed-community sample transfer), StC (static incubations with mixed-community sample transfer), and StB (static incubations with biofilm-only sample transfer). For each community replicate microcosms (n=8) were established; one was used for the next transfer and the others sacrificed for assays.

Figure S2. Development of the high-O_2 region in static microcosms. The development of O_2 gradients in the liquid column of static microcosms can be visualized with Methylene blue which is colourless in the absence of O_2. Shown here are a series of images taken of a static microcosm established using a soil-wash (SW) inoculum and incubated statically for 24h. The development of the high-O_2 region is detectable within 3h (white arrow), but as the community develops the Methylene blue colour is hidden and it is no longer visible after 24h. Once shaken, the 24 h microcosm briefly colours before the O_2 is once again removed by the metabolic activity of the community (last image on the right).

Figure S3. Community productivity varies across serial transfers and treatments. Replicate communities established with soil-wash inocula were serially-transferred ten times across eleven microcosms with productivity determined by OD_{600} measurements for nine treatments (Panels A – I; one, three and six-day ShC (shaken incubation with mixed community sample transfer), StC (static incubation with mixed-community sample transfer) and StB (static incubation with biofilm-only sample transfer) final-transfer communities). Productivities are shown in panels arranged into columns according to the incubation period between transfers and rows according to incubation conditions and transfer sample type. Community replicates (n=3; black, grey and white circles) are shown as the median and range of the microcosm replicates (n=7). A boxplot centred on the median is shown next to each set of community replicates to indicate the pooled community data (n=21). Significant differences between microcosm transfers were observed for all treatments (Kruskal-Wallis, P<0.05).

Figure S4. Media age limits productivity in microcosms. Communities incubated in KB* microcosms reduce available nutrients and accumulate toxic metabolites over time which

limits further growth. Growth (OD_{600}) of the model pseudomonad, *P. fluorescens* SBW25 (Panel A), becomes progressively limited as fresh KB* is diluted with water (black circles) or with cell-free (aged) culture supernatant (white circles) in shaken test microcosms. This effect is dependent on the age of the culture media (Panel B) and effects the growth of SBW25 (white circles) as well as soil-wash (SW) inocula (grey circles) and six-day StB (static incubation with biofilm-only sample transfers) community samples (black circles). Data are shown here as means with standard errors of the mean (n=5) but for clarity the error bars are not shown where they are smaller than the mean symbol. Means not linked by the same letter are significantly different (TK-HSD, α=0.05).

Figure S5. Changes in community diversity. Differences in community diversity between the initial soil-wash (SW) inoculum and the six-day final-transfer communities were assessed by characterising strain phenotypes using a variety of growth-based and behavioural assays and the combined biofilm assay (CBA) for 24 strains isolated from the SW inoculum and each of the six-day ShC (shaken incubation with mixed community sample transfer), StC (static incubation with mixed-community sample transfer) and StB (static incubation with biofilm-only sample transfer) final-transfer communities. Shown are the Inverse Simpson's (1/D) (Panel A) and Shannon Diversity (H) (Panel B) indices determined from the growth-based and behavioural assays (black circles) and the CBA (white circles). Data are shown here as means with standard errors of the mean (n=3; three sets of eight strains from the SW and eight strains from each of the three community replicates for the ShC, StC and StB treatments) but for clarity the error bars are not shown where they are smaller than the mean symbol. Means not linked by the same letter are significantly different (indicated in black for the strain phenotypes and in grey for the CBA data; TK-HSD, α=0.05; trends are suggested by dashed lines).

Figure S6. Competitive and antagonistic interactions between strains. Pairwise interactions between strains isolated from the soil-wash (SW) inoculum (n=24, 'test' strains) and the six-day ShC (shaken incubation with mixed community sample transfer), StC (static incubation with mixed-community sample transfer) and StB (static incubation with biofilm-only sample transfer) final-transfer communities (n=12 from each, 'spot' strains) were assessed using plate-based assays. In these tests, competitive interactions resulted in reduced growth or one or both strains, antagonistic interactions resulted in growth suppression of one or both strains, while neutral interactions did not affect the growth of either strain. Shown here are total antagonistic (white circles), competitive (grey circles) and neutral (white circles) interactions and a Chi-square test of independence indicates that strain origin (ShC, StC & StB) and interaction type (antagonistic, competitive and neutral) were dependent (X^2, P=0.01; trends are suggested by dashed lines).

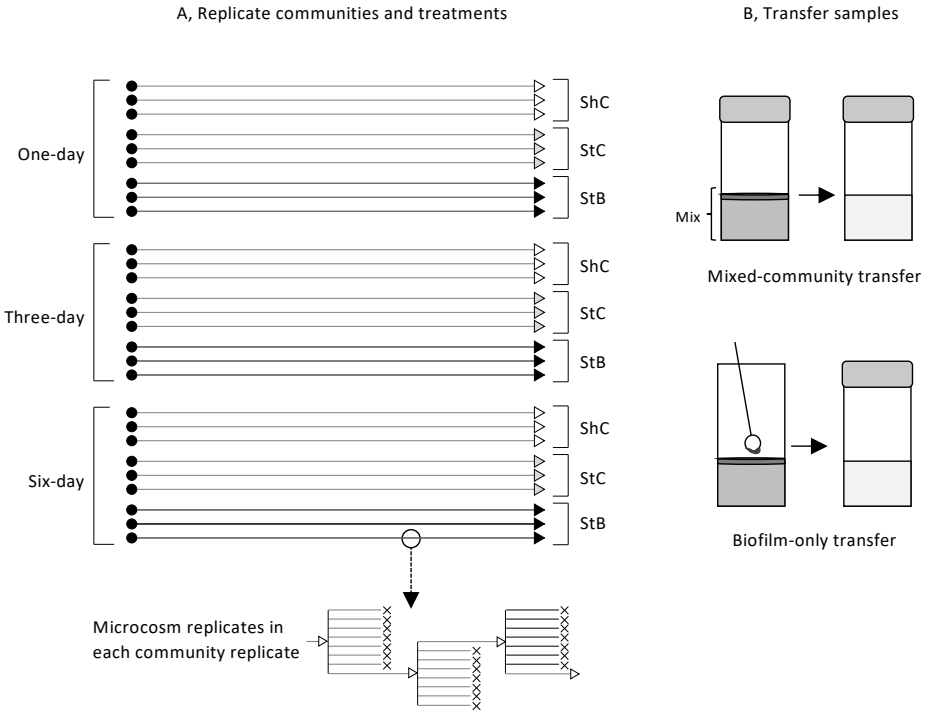
Figure S7. Community enrichment at the top of the liquid columns and mid-column distributions. Static test microcosms inoculated with soil-wash (SW) inoculum and six-day ShC (shaken incubation with mixed community sample transfer), StC (static incubation with mixed-community sample transfer) and StB (static incubation with biofilm-only sample transfer) final-transfer community samples were used to compare cell-distributions (relative OD_{600}) at the top of the liquid column (black circles) and mid-column (white circles) after 24 h incubation. Six samples were taken sequentially from the top of the liquid column which includes the A-L interface biofilm down to the bottom which includes the remains of the cell pellet used to inoculate the microcosm. Data are shown here as means with standard errors of the mean (n=5) but for clarity the error bars are not shown where they are smaller than the mean symbol. Mean mid-column relative OD_{600} was calculated from Samples 2–5. Means not linked by the same letter are significantly different (indicated in black for the top and in grey for mid-column region; TK-HSD, α=0.05; trends are suggested by dashed lines).

Figure S8. Cell-distributions of strains down through the liquid column of static microcosms. Static test microcosms inoculated with representative strains recovered from the soil-wash (SW) inoculum and six-day ShC (shaken incubation with mixed community sample transfer), StC (static incubation with mixed-community sample transfer) and StB (static incubation with biofilm-only sample transfer) final-transfer communities show differences in cell distributions (OD_{600}) after 24 h down through the liquid column (representative strains, n=4, are indicated by black, dark and light grey, and white circles) (Panels A–D). Six samples were taken sequentially from the top of the liquid column which includes the A-L interface biofilm, down to the bottom which includes the remains of the cell pellet used to inoculate the microcosm. Data are shown here as means with standard errors of the mean (n=5) but for clarity the error bars are not shown where they are smaller than the mean symbol. Means of enrichment (top sample) not linked by the same letter within panels are significantly different (TK-HSD, α=0.05; trends are suggested by dashed lines).

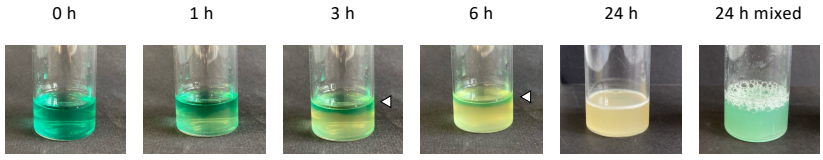
Figure S9. Community members are able to migrate between the top and mid-column regions of the liquid column. Transplant tests were used to determine whether strains could migrate between the top and lower parts of the liquid column. Samples were taken from the A-L interface biofilm (black circles) and mid-region of the liquid column (white circles) of static test microcosms inoculated with the soil-wash (SW) inoculum and 6-D six-day ShC (shaken incubation with mixed community sample transfer), StC (static incubation with mixed-community sample transfer) and StB (static incubation with biofilm-only sample transfer) final-transfer community samples, and transplanted to fresh microcosms and incubated statically before productivity (OD_{600}) (Panel A) and biofilm strength (g) (Panel B) were

assayed after three days. Data are shown here as means with standard errors of the mean (n=5) but for clarity the error bars are not shown where they are smaller than the mean symbol. Means not linked by the same letter within panels are significantly different (TK-HSD, α=0.05; trends are suggested by dashed lines except for biofilm strength for the A-L interface biofilm transplants).

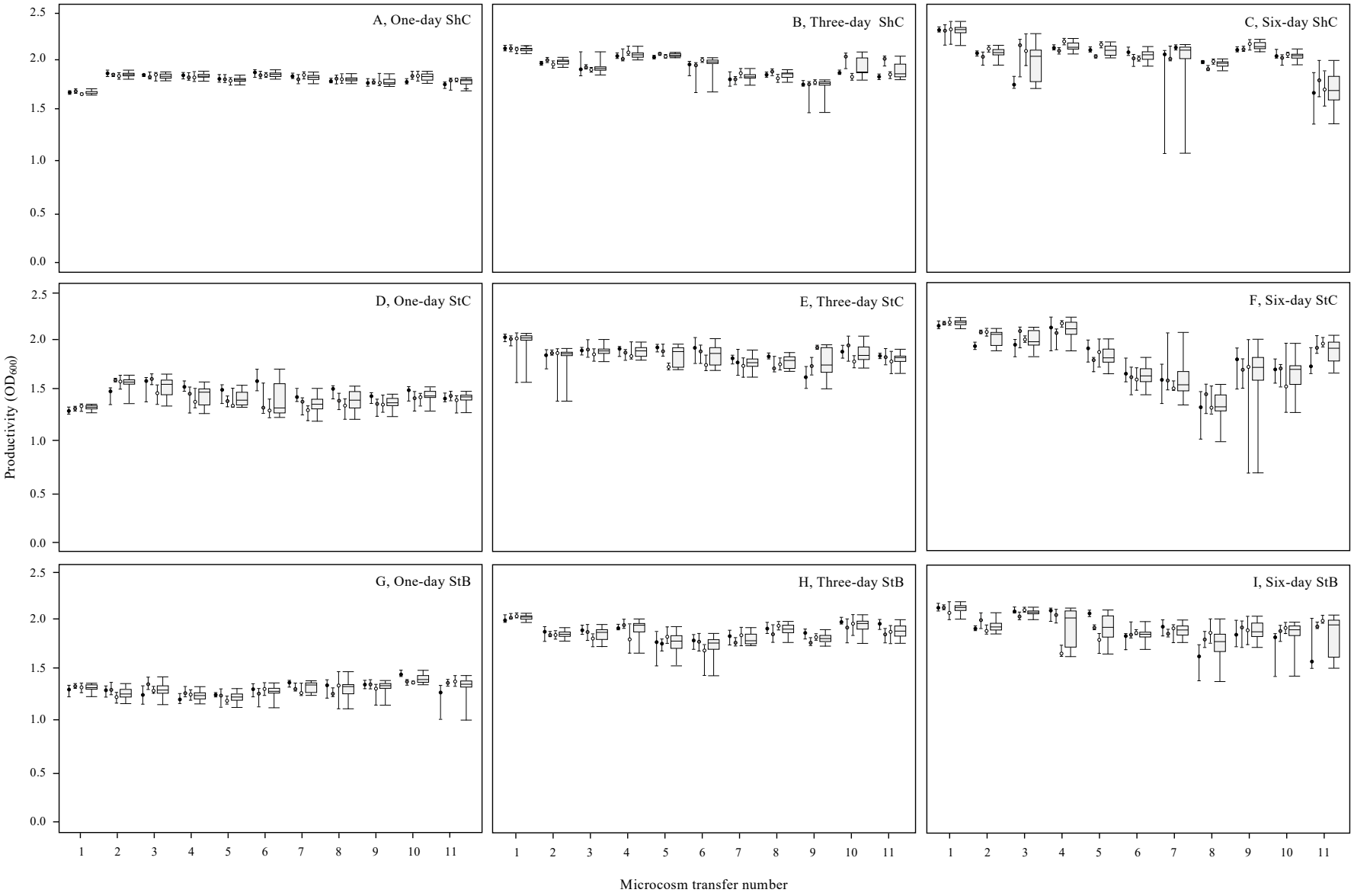
Figure S10. Productivity of communities and strains are affected by oxygenation levels. The productivity of the soil-wash (SW) inocula and six-day final-transfer community samples and individual strains were assessed in test microcosms which provided different oxygenation levels (very-low, normal or high levels) after 24 h incubation. Productivity (OD_{600}) under high-O_2 conditions is compared to productivity under normal conditions (Panel A), and productivity under normal conditions is compared to productivity under very-low O_2 conditions (Panel B). Productivity of soil-wash and community samples are indicated with diamonds and the productivity of individual strains (n=8 for each of the soil-wash and final-transfer communities) by circles. Community samples and strains are shown using the same colours (black, soil-wash inocula; and dark grey, ShC (shaken incubation with mixed community sample transfer); light grey, StC (static incubation with mixed-community sample transfer); and white, StB (static incubation with biofilm-only sample transfer) final-transfer community samples and strains. The dashed lines indicate where productivities are equal for each pairwise comparison of oxygenation levels. Data are shown here as means with standard errors of the mean (n=3) but for clarity the error bars are not shown as they are smaller than the mean symbols.



Supplementary Figure S1



Supplementary Figure S2



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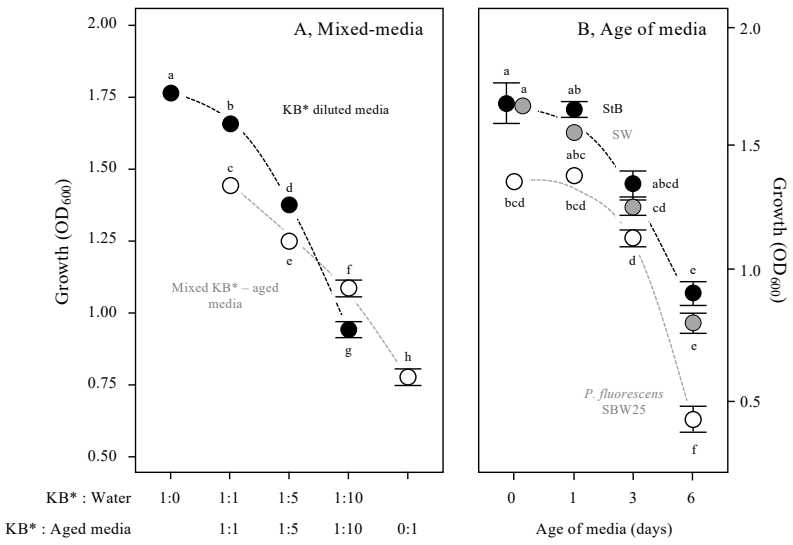

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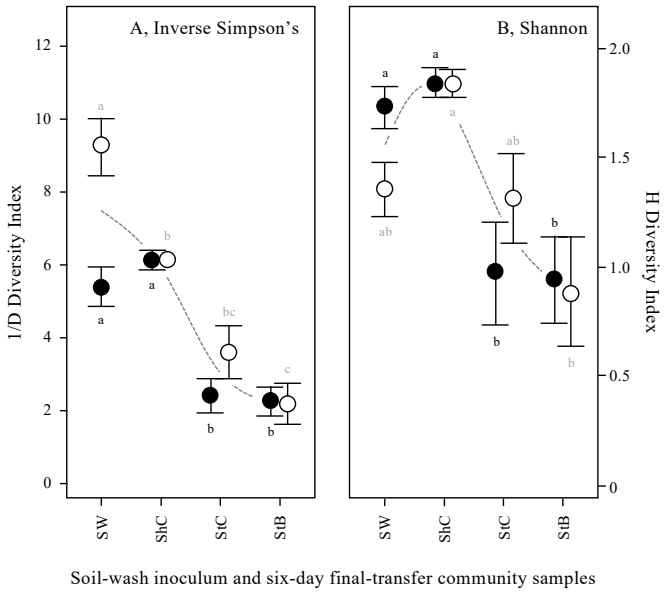

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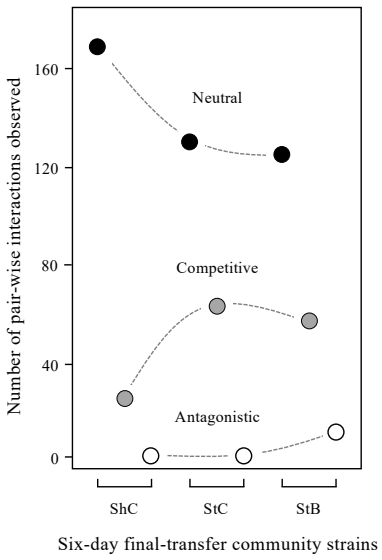

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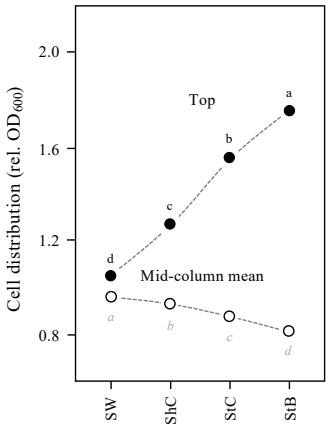

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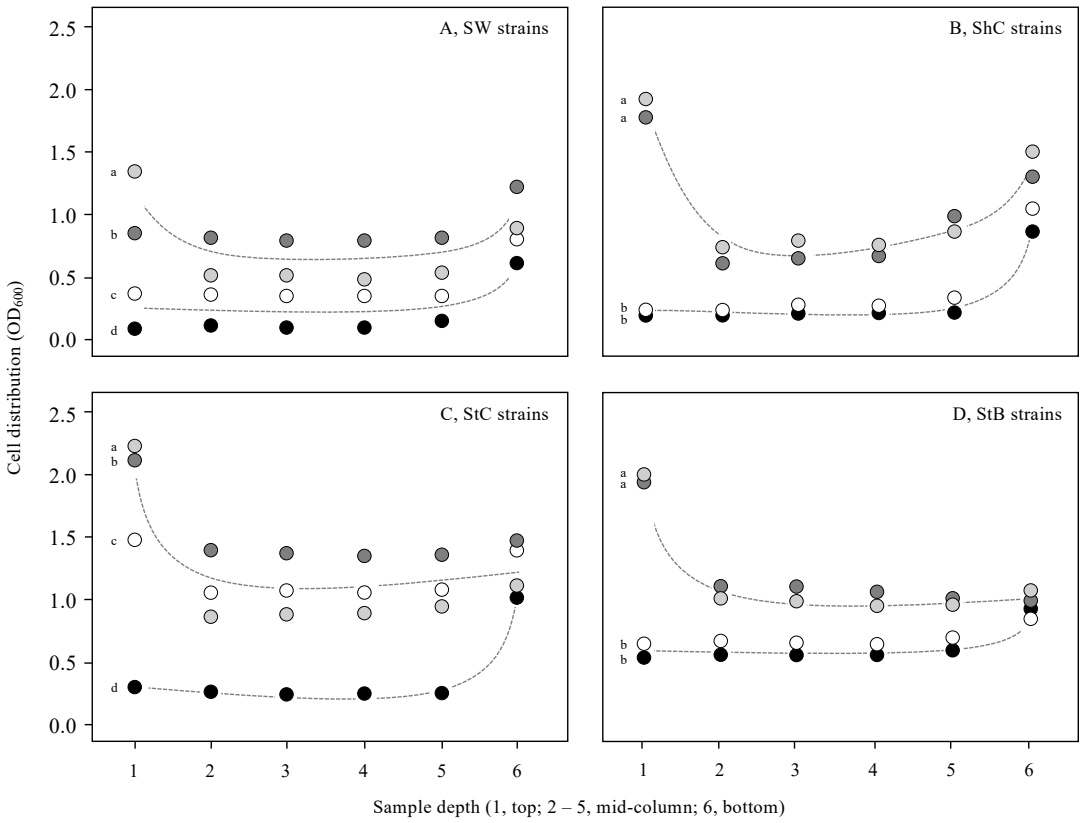

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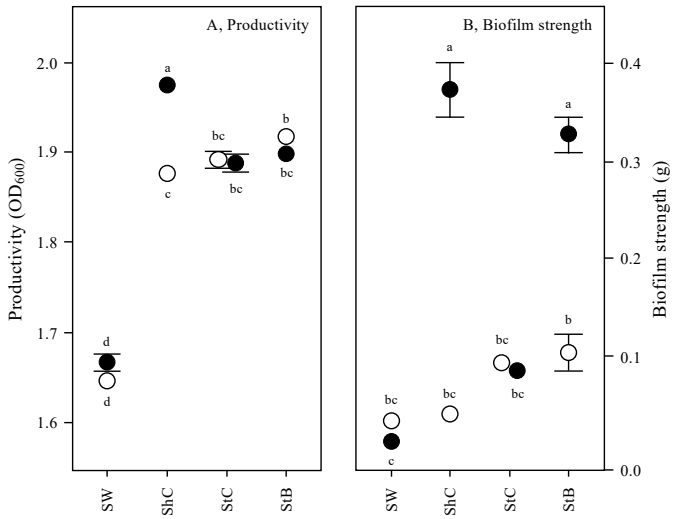
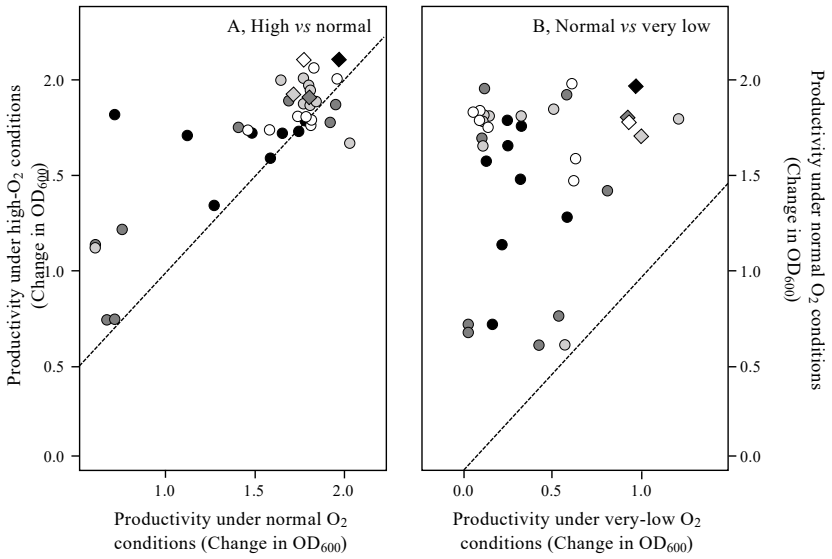


Soil-wash inoculum and six-day final-transfer community biofilm and mid-column transplant samples

Supplementary Figure S9



Supplementary Figure S10